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The effects of a single intravenous injection of novel activin A/BMP-2 (AB204) on toxicity and the respiratory and central nervous systems

Byung-Hak Yoon^{1,2}, Jae Hyup Lee³, Kyuheum Na⁴, Chihoon Ahn¹, Jongho Cho⁴, Hyun Chan Ahn⁴, Jungyoun Choi¹, Hyosun Oh¹, Byong Moon Kim⁴, and Senyon Choe^{1,5}

¹Protein Engineering Laboratory, Joint Center for Biosciences at Songdo Smart Valley, Incheon, Republic of Korea

²Department of Basic Science, Hongik University, Mapo-Gu, Seoul, Republic of Korea

³Department of Orthopedic Surgery, College of Medicine, Seoul National University, SMG-SNU Boramae Medical Center, Seoul, Republic of Korea

⁴Dong-a Socio Holdings Research Institute, Giheung-Gu, Yongin, Gyeonggi, Republic of Korea

⁵Drug Discovery Collaboratory, Qualcomm Institute, University of California San Diego, La Jolla, CA, U.S.A

Abstract

The purpose of this study was to determine the effects of a single intravenous injection of a novel osteoinductive material, activin A/BMP-2 (AB204), to rodents on toxicity and their respiratory functions and central nervous system (CNS). A single intravenous injection of AB204 was given to Sprague–Dawley (SD) rats in doses of 0, 0.625, 2.5 and 10 mg/kg to observe the mortality rate, the general symptoms for 14 days. The experimental groups were also given 0.2, 0.4 and 0.8 mg/kg of AB204, respectively, and the respiration rate, the tidal volume and the minute volume were measured for 240 min. The experimental groups of imprinting control region (ICR) mice were given a single intravenous injection of 0.2, 0.4 and 0.8 mg/kg of AB204, respectively. Their body temperature was taken and general behaviors were observed to evaluate the effect of AB204 on the CNS for 240 min.

The study on toxicity of a single intravenous injection found no death or abnormal symptoms, abnormal findings from autopsy, or abnormal body weight gain or loss in all the experimental groups. No abnormal variation associated with the test substance was observed in the respiration rate, the tidal volume, the minute volume, body temperature or the general behaviors. On the basis of these results, the approximate lethal dose of AB204 for a single intravenous injection exceeds 10 mg/kg for SD rats and a single intravenous injection of 0.8 mg/kg AB204 has no effect on their respiratory system for SD rat and no effect on their CNS for ICR mice.

Address for correspondence: Jae Hyup Lee, Department of Orthopedic Surgery, College of Medicine, Seoul National University, SMG-SNU Boramae Center, 41, Boramae-Gil, Dongjak-Gu, Seoul, 156-707, Korea. Tel: +82-2-870-2314. Fax: +82-2-870-3863. spinelee@snu.ac.kr.

Declaration of interest

All authors disclose here that there are no conflicts of interest that could inappropriately have influenced the outcome of the present study.

Keywords

Activin A; BMP-2; central nervous system; respiratory function; toxicity

Introduction

An increasing population of aged patients has led to more surgical procedures requiring bone grafting and the use of osteoinductive bone graft substitutes is on the increase due to the limited amount of autologous bone grafts or the poor quality of autologous bones or with the aim of avoiding complications during the process of harvesting autologous bones and of seeing more effective bone healing (McKay et al., 2007; Oh et al., 2015). Recombinant human bone morphogenetic protein-2 (rhBMP-2) is known as the most effective bone graft substitute currently in use and is widely employed in spinal, long bone and maxillofacial surgery (Sandhu, 2003; Schuckert et al., 2011; Tressler et al., 2011). rhBMP-2 belongs to TGF- β superfamily and reportedly develops various types of complications, including ectopic bone formation, soft tissue swelling (Smucker et al., 2006), retrograde ejaculation, osteolysis (Knox et al., 2011), radiculopathy (Owens et al., 2011) and seroma formation. The most serious complication is increased cancer risk (Carragee et al., 2011), which is still controversial: Fu et al. (2013) observed that rhBMP-2 increased cancer risk in spinal fusion, whereas Simmonds et al. (2013) found no definite correlation between rhBMP-2 and increased cancer risk. Such a complication is associated with a relatively high dose of rhBMP-2, which ranges from several milligrams to 10 mg or more, in such surgical procedures for a human body as lumbar spinal fusion (Lee et al., 2014). It is therefore desirable to use a low dose of rhBMP-2 to reduce its complications. For this reason, studies have been conducted on growth factors, a low dose of which can generate an effect similar to, or better than, osteoinductivity of rhBMP-2.

Novel activin A/BMP2 (AB2) chimera, AB204 is known to promote osteogenesis and to be more effective in bone healing than rhBMP-2 (Yoon et al., 2014). AB204 even in a 10-fold lower dose was significantly better at bone healing than rhBMP-2 in a mice calvarial defect model and in a tibial defect model (Yoon et al., 2014). However, no research has mentioned its toxic effect on the respiratory or CNS.

This study aims to assess general toxicity of a single intravenous injection of AB204 and measure the respiration rate and the tidal volume to determine its effects on the respiratory and CNS.

Materials and methods

Materials

The test substance, activin A/BMP-2 (AB204), had a form of lyophilized white powder with 95% purity and was kept in a deep freezer.

The excipient in the toxicity study was 50 mM sodium acetate buffer (pH 5.0), which was composed of sodium acetate anhydrate (Sigma-Aldrich, St. Louis, MO acetic acid (Samchum chemical, Pyeongtaek Si, South Korea), and sterile saline (Dai Han Pharm. Co.,

Ltd., Seoul, South Korea). In the toxicity study, 10 mg/kg of AB204 was completely dissolved by vortexing into a suitable amount of 50 mM sodium acetate buffer to prepare a 10 mg/kg AB204 specimen that met the final volume and 2.5 mg/kg and 0.625 mg/kg specimens were prepared through step-by-step dilution with the excipient. The preparation was performed just before the administration and the 10 mg/kg AB204 specimen had pH 5.11.

Sterile saline (Dai Han Pharm. Co. Ltd., Seoul, South Korea) was used as an excipient to test the functions of the respiratory and CNS and pH of the test substance was estimated to be 5.1 and 5.0, respectively, in the high-dose groups.

Animals

The toxicity study involved 48 specific pathogen-free (SPF) SD (Hds: SD[®]) rats (Koatech., Gyunggi-do, Korea) (24 males, 197.0–216.8 g; 24 females, 140.3–163.4 g) and the respiratory function test used 35 male SD (Crl: CD (SD)) rats (Koatech., Gyunggi-do, Korea) (115.7–132.0 g). 35 SPF (Hds: CD-1) imprinting control region (ICR) mice (Koatech., Gyunggi-do, Korea) (28.8–32.7 g) were admitted to test the functions of the CNS.

When admitted, all animals were inspected visually and their body weight was measured with an electronic scale (CP3202S, Sartorius, Germany). During 7 days of acclimation period, clinical observations were made every day. At the end of the acclimation period, their body weight was measured and general symptoms and body weight variation were checked in order to make sure that all of them were in normal conditions. A marker was used to mark their tail in red during the acclimation period and in black during the observation period and each of the cages was given an identification number. Body weight ranking was decided for the animals found to be healthy during the acclimation period and they were randomly grouped so that the groups could be almost equal in the mean body weight (Table 1).

The rearing conditions were as follows: 22.6 °C to 23.8 °C for temperature, 51.9% to 63.0% for relative humidity, 10 to 20 ventilations per h, 12 -h/day of light/dark cycle (from 8 a.m. to 8 p.m.), and 150 to 300 Lux for illumination intensity. The animals were let to freely have solid feed for laboratory animals—Teklad certified irradiated global 18% protein rodent diet (2918C, Harlan Laboratories Inc., Indianapolis, IN) – and to freely drink ultraviolet-irradiated and filtered underground water from a bottle. Three or fewer SD rats were put in each wire mesh cage with stainless steel and one ICR mouse was put in each polycarbonate cage.

Test substance administration

Test substance administration in toxicity study—About 10 mL/kg of test substance was injected into the tail vein at the speed of 4 mL/min on the basis of the body weight measured on the injection day.

Study design

This study observed the Good Laboratory Practice (GLP) provisions and was based on Notification No. 2009-102 of the Korea Food and Drug Administration (KFDA), which is a nonclinical test guideline, and on the toxicity test guideline for drugs and others (KFDA Notification No. 2009-116). It was approved by the Institutional Animal Care and Use Committee in the Nonclinical research Institute of ChemOn Incorporation (Gyeonggi-do, Korea) based on the Animal Protection Act (serial number: 12-R021 for the toxicity study, 12-R316 for the study on the respiratory functions, and 14-M139 for the study on the functions of the CNS).

Methods

Methods for toxicity study

General symptoms: All the SD-rats were observed constantly within one hour after the administration and on an hourly basis within six hours after that on the administration day and observation was made once a day or more frequently between one day and 14 days after the administration. The administration day was set at Day 0.

Measuring body weight: Body weight was measured for all the SD-rats before administration (when they were admitted and grouped) and on Days 0, 1, 3, 7 and 14.

Autopsy: On Day 14, all the live SD-rats were killed by venesection, which involved cutting their abdomen open and cutting vena cava and abdominal aorta, under anesthesia using CO₂; then, the autopsy findings concerning the body surface and the intestines were inspected visually. The administration sites of all the live animals were put in a 10% neutral-buffered formalin solution.

Methods for study on respiratory functions

Observation of general symptoms: All the SD-rats were made to adapt themselves to the environment inside the chamber of the respirometer (Buxco Electronics, Inc., Wilmington, NC) to measure the respiration rate and the tidal volume for about 30 minutes on a daily basis from the admission day until the day before administration. All the SD-rats were observed for general symptoms once or more on Day 0.

Measurement of respiration rate and tidal volume: During the measurement period, two SD-rats in each group were measured in numerical order on a daily basis. Their body weight was measured to estimate the injection volume on the day of test. They were fully relieved within the chamber of the respirometer (Buxco Electronics, Inc., Wilmington, NC) to measure the respiration rate and the tidal volume before injecting the test substance; when their breath became stable, the respiration rate, the tidal volume, and the minute volume were measured for 10 min.

For each SD-rat, the respiration rate per minute, the tidal volume, and the minute volume were measured for 10 min at 15, 30, 60, 120 and 240 min after administering the test substance.

Methods for study on functions of central nervous system—Nineteen items on functions of CNS of each ICR mouse were measured and observed before administering the test substance and 15, 30, 60, 120 and 240 min after the administration (Table 2).

Statistical analysis

For statistical analysis, SPSS 10.1 K, a statistical package commercially in wide use, was used, with the significance level set at $p < 0.05$. In the toxicity study, body weight and body weight gain were shown in the mean and standard deviation and parametric one-way analysis of variance (ANOVA) was employed, assuming normality of the data. Levene's test was performed to determine the homogeneity of variance. In the study on the respiratory functions, parametric one-way ANOVA was employed, assuming normality of the data about the respiration rate, the tidal volume and the minute volume. Levene's test was performed to determine homogeneity of variance.

In the study on the functions of the CNS, parametric oneway ANOVA was employed, assuming normality of the data about body temperature. Levene's test was performed to determine homogeneity of variance.

Results

Results of toxicity study in SD rats

General symptoms—No case of death or abnormality of general symptoms was found in both male and female SD rats of any group during the observation period.

Body weight variation and feed intake—No significant variation was found in body weight in both male and female SD rats of any group before test substance administration. The final follow-up found significant weight gain in both male and female SD rats of all the groups, including the control group, as compared with before test substance administration ($p < 0.0001$ in all cases). However, no significant difference was found in body weight between each experimental group and the control group during the observation period or in the final follow-up (Table 3).

Autopsy findings—No abnormal finding was observed in both male and female SD rats of any experimental group.

Results of study on respiratory functions in SD rats

General symptoms—No abnormal finding associated with test substance was observed during the observation period.

Respiration rate—No variation associated with the test substance was observed during any measurement period (Table 4).

Tidal volume—No variation associated with the test substance was observed during any measurement period (Table 5).

Minute volume—No variation associated with the test substance was observed during any measurement period (Table 6).

Results of study on functions of central nervous system in ICR mice

Body temperature—No body temperature variation associated with the test substance was observed during any measurement period (Table 7).

General behavior—Normal findings were observed during all the periods in terms of catalepsy, traction, tremors, convulsion, exophthalmos, piloerection, salivation, lacrimation, diarrhea, skin color, pinna reflex, righting reflex, tail elevation, palpebral closure, the abdominal tone, locomotion, the respiration rate and death.

Discussion

Activin A/BMP2 (AB204) chimera, AB204 in this study, is known to be more effective in osteoblastic differentiation and in bone healing than rhBMP-2 (Allendorph et al., 2011; Yoon et al., 2014). That is, it can obtain bone healing equal to or better than that of rhBMP-2 even in a significantly lower dose and, consequently, can lead to lower frequency and levels of adverse events. The higher effectiveness of AB204 in bone healing than rhBMP-2 is associated with a receptor for binding. That is, both rhBMP-2 and activins are dimeric TGF- β superfamily ligands that are bound with type I and type II transmembrane serine/threonine receptors kinases and assemble them to give signals (Yoon et al., 2014). BMP-2 has high affinity to type I receptors Alk1, Alk2, Alk3, and Alk6 and activates Smads 1, 5, and 8 pathway (Chen et al., 1997; Hoodless et al., 1996; Nishimura et al., 1998), whereas activins has high affinity to type II receptors ActRII and ActRIIb and activates Smads 2 and 3 (Gray et al., 2000; Massague & Weis-Garcia, 1996; Zimmerman & Mathews, 1996). It is expected, therefore, that activin A/BMP2 (AB2) chimera will have better BMP2-like signaling properties than rhBMP-2 alone (Yoon et al., 2014).

Since we grafted a total of 100 μ g – 50 μ g for each side – at one level and got a satisfactory fusion rate in a lumbar posterolateral fusion model using a beagle dog, it is expected that about 500 μ g is applicable to human beings. In this case, a single intravenous injection of the high dose of AB204 (10 mg/kg) in this study is equivalent to a 1200-fold higher dose than actual one for a human being weighing about 60 kg.

The single intravenous administration of such a high dose of AB204 to SD rats generated no death and made no abnormal variation in the general symptoms, body weight and the autopsy findings. It is believed, therefore, that the approximate lethal dose (ALD) of the test substance, AB204, for a single intravenous injection to SD rats exceeds 10 mg/kg, regardless of sex, under these experimental conditions.

In all the experimental groups, a single intravenous administration of AB204 to SD rats made no abnormal variation in the respiration rate or in the tidal volume. This result implies that a single intravenous administration of 0.8 mg/kg of the test substance, AB204, to SD rats may have no effect on their respiratory system under these experimental conditions.

A single intravenous administration of AB204 to ICR mice made no abnormal variation in their body temperature or general behaviors in any experimental group. This result implies that a single intravenous administration of 0.8 mg/kg AB204 to ICR mice may have no effect on their CNS under these experimental conditions.

This study has some limitations. It cannot predict toxicity of repetitive exposure to AB204 due to the absence of findings about toxicity of repetitive administration and it made no mention of suspected carcinogenesis in rhBMP-2. Nevertheless, the results that ALD of AB204 for SD rats exceeded 10 mg/kg and that 0.8 mg/kg of it had no effect on their respiratory system or on the CNS of ICL mice are expected to be basic data in designing a study on repetitive administration.

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Table 1

Grouping and injection volumes.

Group	Dosage (mg/kg)	Volume (mL/kg)	Number of animals	
			male	female
Toxicity study (SD rats)				
G1 (control group)	0	10	5	5
G2 (low-dose group)	0.625	10	5	5
G3 (intermediate dose group)	2.5	10	5	5
G4 (high-dose group)	10	10	5	5
Respiratory function study (SD rats)				
G1 (control group)	0	10	8	
G2 (low-dose group)	0.2	10	8	
G3 (intermediate dose group)	0.4	10	8	
G4 (high-dose group)	0.8	10	8	
CNS function study (ICR mice)				
G1 (control group)	0	5	8	
G2 (low-dose group)	0.2	5	8	
G3 (intermediate dose group)	0.4	5	8	
G4 (high-dose group)	0.8	5	8	

SD rats: Sprague–Dawley rats.

ICR mice: Imprinting control region mice.

Table 2

Items on function of central nervous system of ICR mice.

Items	Methods for study
Body temperature	Body temperature within the rectum was taken
Catalepsy	Each mouse was stood upside down by a cylinder (10 cm in diameter), in order to observe the movement of four feet within 8 sec
Traction	Each mouse was put on an iron rod 30 cm in height to see whether it kept clinging to the rod for 5 sec or more
Tremor	Each mouse was observed for tremor
Convulsion	Each mouse was observed for convulsion
Exophthalmos	Each mouse was observed for exophthalmos
Piloerection	Each mouse was observed for piloerection
Salivation	Each mouse was observed for salivation
Lacrimination	Each mouse was observed for lacrimination
Diarrhea	Each mouse was observed for diarrhea
Skin color	For each mouse, the color of the ears and the tail was determined
Pinna reflex	Each mouse was observed for pinna reflex
Righting reflex	The body of each mouse was turned to see whether it resumed good posture immediately
Tail elevation	The level of tail elevation was determined if it put its tail on a floor
Palpebral closure	The level of palpebral closure was determined if its eyes were wide open as usual
Abdominal tone	The level of abdominal tone was determined if abdominal muscle was completely relaxed on the basis of palpation
Locomotion	Each mouse was observed for locomotion
Respiration rate	The respiration rate of each mouse was checked
Death	Each mouse was examined for death

ICR mice: Imprinting control region mice.

Table 3

Body weight of single intravenous dose toxicity study in SD rats.

Sex	Group/Dose (mg/kg)	Day after treatment, g (mean $\pm$ SD)					
		0	1	3	7	14	
Male	G1 (0)	259.1 $\pm$ 8.3	264.5 $\pm$ 6.5	269.9 $\pm$ 8.1	297.3 $\pm$ 14.3	332.1 $\pm$ 23.1	
	G2 (0.625)	266.2 $\pm$ 11.6	271.5 $\pm$ 10.7	277.0 $\pm$ 10.2	306.2 $\pm$ 9.4	341.9 $\pm$ 10.8	
	G3 (2.5)	265.9 $\pm$ 7.9	269.2 $\pm$ 9.5	275.5 $\pm$ 9.1	303.2 $\pm$ 14.1	338.2 $\pm$ 19.4	
	G4 (10)	261.0 $\pm$ 8.2	262.6 $\pm$ 8.9	272.4 $\pm$ 8.7	303.2 $\pm$ 12.4	344.0 $\pm$ 17.9	
Female	G1 (0)	171.4 $\pm$ 8.8	174.0 $\pm$ 9.4	179.0 $\pm$ 7.2	191.4 $\pm$ 8.8	208.5 $\pm$ 9.2	
	G2 (0.05)	171.5 $\pm$ 5.9	173.3 $\pm$ 8.2	178.2 $\pm$ 5.4	190.9 $\pm$ 6.8	213.4 $\pm$ 6.4	
	G3 (0.18)	171.4 $\pm$ 6.8	174.5 $\pm$ 8.1	178.4 $\pm$ 5.7	191.1 $\pm$ 3.7	212.3 $\pm$ 9.4	
	G4 (0.5)	174.0 $\pm$ 5.3	175.7 $\pm$ 7.3	179.1 $\pm$ 10.4	190.1 $\pm$ 10.3	210.6 $\pm$ 12.7	

The number of SD rats of each group was 5.

No significant difference was found in body weight between each experimental group and the control group.

SD: standard deviation.

Table 4

Respiration rate in SD rats.

Respiration rate (breaths/min)		Time (min)					
		0	15	30	60	120	240
Group	Dose (mg/kg)	SD rats No.					
G1	0	8	130.6 ± 23.5	118.5 ± 13.8	142.9 ± 33.7	105.2 ± 11.0	117.5 ± 22.4
G2	0.2	8	131.6 ± 21.1	130.3 ± 38.6	117.8 ± 21.5	116.5 ± 22.9	109.9 ± 26.5
G3	0.4	8	116.5 ± 17.2	108.8 ± 16.8	119.8 ± 30.0	116.9 ± 28.5	108.2 ± 26.3
G4	0.8	8	128.1 ± 26.8	126.1 ± 32.7	132.8 ± 25.9	130.6 ± 36.3	115.9 ± 23.5

No variation associated with the test substance was observed.

Table 5

Tidal volume in SD rats.

Tidal volume (mL)			Time (min)					
Group	Dose (mg/kg)	SD rats No.	0	15	30	60	120	240
G1	0	8	1.28 ± 0.17	1.36 ± 0.16	1.39 ± 0.12	1.40 ± 0.12	1.26 ± 0.17	1.36 ± 0.13
G2	0.2	8	1.44 ± 0.12	1.34 ± 0.08	1.38 ± 0.08	1.40 ± 0.08	1.38 ± 0.11	1.33 ± 0.16
G3	0.4	8	1.41 ± 0.12	1.36 ± 0.12	1.39 ± 0.11	1.41 ± 0.09	1.41 ± 0.09	1.33 ± 0.11
G4	0.8	8	1.34 ± 0.17	1.35 ± 0.10	1.40 ± 0.11	1.39 ± 0.11	1.32 ± 0.17	1.33 ± 0.10

No variation associated with the test substance was observed.

Table 6

Minute volume in SD rats.

Tidal volume (mL)								
Time (min)								
Group	Dose (mg/kg)	SD rats No.	0	15	30	60	120	240
G1	0	8	157.2 ± 21.8	150.1 ± 12.3	192.7 ± 62.4	140.5 ± 11.1	142.5 ± 32.1	134.3 ± 12.5
G2	0.2	8	179.6 ± 27.3	162.5 ± 38.7	154.0 ± 26.1	152.5 ± 24.7	142.1 ± 24.5	131.1 ± 20.7
G3	0.4	8	157.6 ± 29.9	141.0 ± 19.7	156.2 ± 36.6	155.9 ± 38.0	143.5 ± 34.3	142.0 ± 43.8
G4	0.8	8	161.7 ± 31.1	159.5 ± 28.5	174.1 ± 30.6	171.2 ± 45.8	143.1 ± 15.8	145.4 ± 27.9

No variation associated with the test substance was observed.

Table 7

Body temperature in ICR mice.

Body temperature (°C)		Time (min)						
Group	Dose (mg/kg)	ICR mice No.	0	15	30	60	120	240
G1	0	8	38.2 ± 0.4	38.7 ± 0.6	38.7 ± 0.5	38.6 ± 0.3	38.4 ± 0.4	37.9 ± 0.2
G2	0.2	8	38.1 ± 0.4	38.4 ± 0.5	38.7 ± 0.4	38.7 ± 0.5	38.2 ± 0.3	38.1 ± 0.3
G3	0.4	8	38.1 ± 0.4	38.8 ± 0.5	38.7 ± 0.5	38.5 ± 0.5	38.2 ± 0.5	38.0 ± 0.2
G4	0.8	8	38.1 ± 0.4	38.3 ± 0.3	38.3 ± 0.5	38.2 ± 0.5	37.9 ± 0.5	37.9 ± 0.3

No body temperature variation associated with the test substance was observed.